Original Article

Mendelian randomization study on the causal relationship between chronic hepatitis B/C virus infection and idiopathic pulmonary fibrosis

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Background: The pathogenesis of idiopathic pulmonary fibrosis (IPF) is not well understood. Given the known role of hepatitis C virus (HCV) in inducing cirrhosis, the virus has also received attention in the study of IPF. An earlier retrospective study found an increased incidence of IPF in patients with HCV, supported by evidence in the alveolar lavage fluid of the patients, whereas another set of observational studies did not find an association, which prompted us to explore a causal relationship. It is well known that HCV and hepatitis B virus (HBV) have some similarities: both are RNA viruses, and both have a strong ability to induce cirrhosis, which in turn leads to poor prognosis and increased mortality in patients with viral hepatitis. This factor also inspired us to start exploring whether there is a causal relationship between HBV and IPF. Due to the inherent limitations of previous studies, causality between chronic HBV/HCV infection and IPF is yet to be established. Mendelian randomization (MR) uses genetic variation as exposure and can be used to determine the causal effect of exposure on outcomes. Therefore, we used a two-sample MR study to determine if there is a causal relationship between viral hepatitis and IPF risk.

Methods: Single nucleotide polymorphisms (SNPs) were used as instrumental variables (IVs), with chronic HBV and HCV infections as exposure factors and IPF as the outcome variable. Three methods, inverse variance weighting (IVW), weighted median (WM), and MR-Egger regression, were employed for the bidirectional MR. Sensitivity analyses, including horizontal pleiotropy analysis, Cochran's *Q* test, and leave-one-out evaluation of result reliability, were conducted. Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) and MR-Egger regression tests were used to monitor potential horizontal pleiotropic effects. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to interpret the causal relationship between chronic HBV and HCV infections and IPF. Finally, reverse MR analysis was performed to validate the robustness of the results.

Results: The results of the IVW suggested that there was no causal relationship between chronic HBV infection (OR =1.039, 95% CI: 0.935–1.154, P=0.48) and chronic HCV infection (OR =1.146, 95% CI: 0.834–1.576, P=0.40) and the risk of IPF. Sensitivity analysis showed no evidence of reverse causation, horizontal pleiotropy, and heterogeneity.

Conclusions: This study, using the bidirectional MR, provides preliminary evidence that chronic HBV

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and HCV infections are not causally related to IPF at the genetic level. However, this conclusion requires support from larger sample sizes in genome-wide association study (GWAS) databases for further MR analysis, and additional clinical studies and animal experiments are needed for validation.

Keywords: Idiopathic pulmonary fibrosis (IPF); hepatitis C virus infections (HCV infections); hepatitis B virus infections (HBV infections); Mendelian randomization (MR)

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a unique form of idiopathic chronic progressive fibrosing interstitial lung disease. It is characterized by irreversible lung tissue destruction due to excessive extracellular matrix deposition and remodeling, leading to fibrous scarring, organ damage, respiratory failure, and eventual mortality (1). Despite the increasing global prevalence and incidence, there is currently no universally recognized most effective treatment for IPF. Thus, elucidating its etiology and implementing early prevention strategies are crucial.

Some studies suggest a certain correlation between hepatitis C virus (HCV) infection and the occurrence of IPF. Ueda *et al.* found that chronic viral hepatitis, particularly infection with HCV, can lead to the development of IPF (2). In the serum of 66 IPF patients, they detected an HCV antibody positivity rate of as high as 28.8%, whereas in the control group, the detection rate was only 3.6%. Similarly, Meliconi *et al.* found consistent results: out of 60 IPF patients, 8 cases (13.3%) tested positive for HCV antibodies, showing a significant difference compared to the control group (0.3%) (3). In a retrospective study, Arase *et al.* found that a small percentage of HCV-infected patients developed IPF (4). Interestingly, Idilman *et al.* found that the total number of neutrophils in bronchoalveolar lavage fluid was significantly higher in patients with chronic hepatitis C than in controls, a result consistent with that reported by Cobben *et al.* (5,6). These findings seem to indicate that the immune mechanism triggered by the virus is the pathogenesis of IPF. HCV may induce alveolar inflammation, leading to pulmonary fibrosis. Arase *et al.* speculated that there may be other mechanisms in the formation of IPF in HCV-positive patients, such as the accumulation of immune complexes in lung tissue or the direct involvement of HCV-RNA (4). However, current research results are to some extent contradictory. In serum samples from 62 IPF patients, Irving *et al.* detected only 1 patient with HCV antibody positivity, and this patient's HCV-RNA was negative (7). In follow-up, Arase *et al.* observed only 15 cases of IPF among 6,150 HCV-infected patients (4). To date, most of these studies have been observational, and many studies have included some unadjusted confounding factors. Therefore, the causal relationship between HCV/hepatitis B virus (HBV) and IPF remains unclear. Furthermore, due to the fact that chronic infection with HBV not only leads to organ fibrosis but also severely affects lung function, exploring the causal relationship between chronic HBV infection and the risk of IPF is of certain importance (8).

Genome-wide association studies (GWAS) have tested millions of genetic variations in the genomes of many individuals to determine the genotype-phenotype associations, playing a crucial role in understanding the mechanisms behind complex diseases in the field of

Highlight box

Key findings

- This study firstly used the latest data and modern analytical methods, specifically the genome-wide association study database and Mendelian randomization, to demonstrate that there is no causal relationship between hepatitis C virus (HCV) and hepatitis B virus (HBV) with idiopathic pulmonary fibrosis (IPF), providing a new approach to investigating the pathogenesis of IPF.

What is known and what is new?

- The virus-induced immune mechanism may be one of the causes of IPF.
- The causality between chronic HBV/HCV infection and IPF is yet to be established.

What is the implication, and what should change now?

- There is no causal relationship between chronic HBV/HCV infection at the genetic level and IPF. It has greatly deepened our understanding of the virus-related immune mechanism inducing IPF.

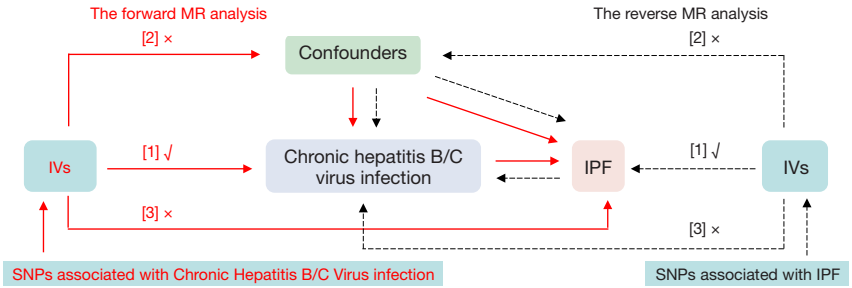

Figure 1 Study design of the two-sample MR analysis on the effect of genetically predicted chronic hepatitis B/C virus infection on IPF. [1], relevance: SNPs robustly associated with exposure; [2], independence: SNPs not associated with confounders; [3], exclusion restriction: SNPs only associated with outcome through exposure. MR, Mendelian randomization; IVs, instrumental variables; SNPs, single nucleotide polymorphisms; IPF, idiopathic pulmonary fibrosis.

genetics (9). Mendelian randomization (MR) study is a method that utilizes genetic variations as instrumental variables (IVs) to infer causal relationships between exposure factors and outcomes in observational studies (10). Genetic variations have inherent characteristics determined at conception, making them less susceptible to confounding factors such as postnatal influences and social environments. Therefore, compared to observational studies and randomized controlled trials, the results of MR studies are more accurate. This study employs a bidirectional MR approach to explore whether two types of viral hepatitis may causally affect the risk of IPF. It also investigates whether the genetic susceptibility to IPF risk may causally influence the two types of viral hepatitis. Based on this, perhaps we can elucidate the roles of the two viral hepatitides in the development of IPF, ultimately contributing to the development of new strategies for prevention and treatment. We present this article in accordance with the STROBE-MR reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-392/rc).

Methods

Study design

This study considered chronic HBV infection and chronic HCV infection as exposure factors, selecting significantly associated single nucleotide polymorphisms (SNPs) as IVs, with IPF as the outcome variable. The causal analysis was conducted using a two-sample MR method, and Cochran's *Q* test was employed to assess heterogeneity in the results. Sensitivity analyses were performed to validate reliability. To choose suitable IVs in the study, three core assumptions were taken, as follows: (I) there is a significant association between IVs and either chronic HBV infection or chronic HCV infection; (II) IVs are unrelated to all confounding factors; (III) IVs do not directly affect the outcome variable but influence the outcome indirectly through their association with the exposure (chronic HBV infection or chronic HCV infection). Finally, to further validate the results, a directional MR analysis was conducted. The study design is illustrated in *Figure 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data for the GWAS and the selection of SNPs

The summary data for GWAS on chronic HBV infection and chronic HCV infection were sourced from the IEU OpenGWAS project. the summarized data for both of the GWAS analyses can be accessed at https://gwas.mrcieu.ac.uk/ (chronic hepatitis C infection GWAS ID: ebi-a-GCST90018805; chronic hepatitis B infection GWAS ID: ebi-a-GCST90018804). These GWAS datasets originate from a 2021 study focusing on the European population (11). Further details can be found in *Table 1*.

The GWAS data for IPF were sourced from the UK Biobank website (https://gwas.mrcieu.ac.uk/), with a study population consisting of individuals of European descent (GWAS ID: finn-b-IPF). From this dataset, we obtained genotype data for 1,028 cases of IPF and 196,986 controls (12,13), along with information on 16,380,413 SNPs.

In this study, IVs were selected from the GWAS summary databases for chronic HBV infection and chronic HCV infection. Firstly, to obtain IVs closely related to the exposure, the threshold for SNPs in IVs was set at P<5e−08. Additionally, all IVs had an *F*-statistic value greater than

Table 1 Brief description of datasets utilized in the Mendelian randomization study

Exposure	Sample size (case/control), n	Number of SNPs	Pleiotropy test		Heterogeneity test	
			Egger_ intercept_P	PRESSO_ Global_P	IVW_ Q_Pval	MR Egger_ Q_Pval
Chronic hepatitis B infection	351,885 (145/351,740)	19,079,722	0.08	0.09	0.11	0.20
Chronic hepatitis C infection	352,013 (273/351,740)	19,074,546	0.65	0.51	0.36	0.26

SNPs, single nucleotide polymorphisms; PRESSO, Pleiotropy RESidual Sum and Outlier; IVW, inverse-variance weighting; MR, Mendelian randomization.

10 [F=(beta/se)^2], effectively mitigating the impact of weak instrument bias in this study (14). Secondly, to ensure the extracted IVs were independent of each other, linkage disequilibrium (LD) coefficients were set at 0.01 (15), and LD block width was set at 1,000 kb, using R software (R version 4.2.3; R Foundation for Statistical Computing, Vienna, Austria) (16). Lastly, this study utilized the effect allele frequency to harmonize the datasets for exposure factors (chronic HBV infection and chronic HCV infection) and the outcome variable (IPF).

Statistical analysis

After harmonizing the IVs for exposure and outcome using the same effect allele, this study employed a random-effects model with the Two Sample MR package (TwoSampleMR version 0.5.8) in R software for two-sample MR analysis. The inverse variance weighting (IVW) method was selected as the primary approach to assess the bidirectional associations between chronic HBV infection, chronic HCV infection, and IPF. Since the IVW method does not account for intercept terms and the presence of pleiotropy, we employed MR-Egger regression and the weighted median (WM) method as complementary validations to address potential limitations of the IVW method. The MR-Egger regression method evaluates potential horizontal pleiotropy through the intercept term. The WM method provides consistent estimates even when 50% of the IV SNPs are invalid. Both of these complementary methods serve to assess the effectiveness and robustness of the results obtained from the IVW method in the study.

To mitigate the impact of bias induced by horizontal pleiotropy and heterogeneity on the study results, this study additionally employed the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test. MR-PRESSO detects horizontal pleiotropy by correcting for it through the removal of outliers, and it tests for significant differences in causal estimates before and after outlier correction. Furthermore, the study used the Cochran Q statistic test to compute heterogeneity in IVW and MR-Egger analyses. In addition, a leave-one-out sensitivity analysis was conducted by iteratively excluding each SNP and calculating the combined effect of the remaining SNPs to assess the individual SNP's impact on the causal relationship and thereby validate the robustness of the results. Finally, all MR analysis results were visually presented through forest plots and scatter plots. As the outcome variable is a binary variable, the study further transformed effect estimates into odds ratios (ORs) for a more intuitive assessment of the relationship between exposure and outcome. All statistical tests were two-sided, and P<0.05 was considered statistically significant.

Results

Table S1 displays summary information for SNPs used as genetic instruments in MR analyses of genetically predicted chronic HBV infection or chronic HCV infection and the risk of IPF. All SNP loci show a statistical strength with F>10, indicating a low likelihood of weak IVs. It is noteworthy that this study does not involve palindromic SNPs and incompatible SNPs.

Based on the random-effects IVW method analysis, this study did not find a significant causal relationship between chronic HBV infection and IPF [OR =1.039, 95% confidence interval (CI): 0.935–1.154, P=0.48], as well as between chronic HCV infection and IPF (OR =1.146, 95% CI: 0.834–1.576, P=0.40) (*Figure 2*).

Horizontal pleiotropy and heterogeneity analysis

Due to differences in sample sources, population ages, and genders, the selected IVs may inevitably exhibit heterogeneity and horizontal pleiotropy in the study. These

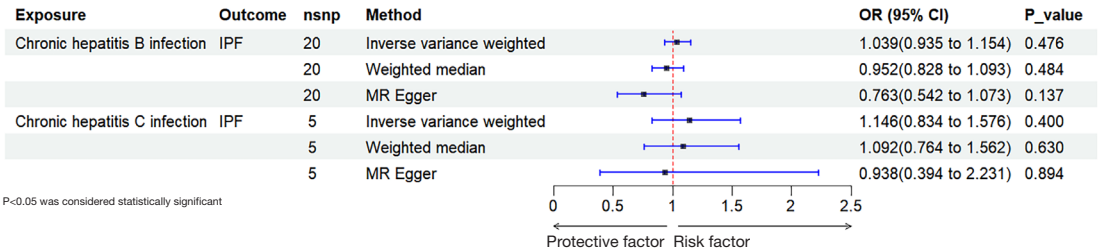

Figure 2 Forest plot of the combined effect of chronic hepatitis B/C virus infection on IPF. IPF, idiopathic pulmonary fibrosis; MR, Mendelian randomization; OR, odds ratio; CI, confidence interval.

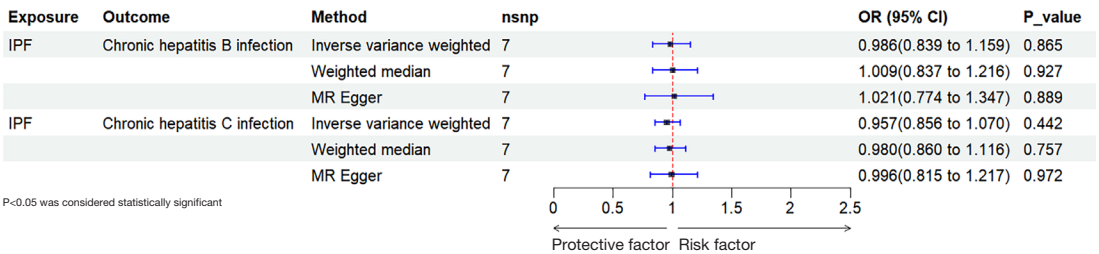

Figure 3 Forest plot of the combined effect of IPF on chronic hepatitis B/C virus infection. IPF, idiopathic pulmonary fibrosis; MR, Mendelian randomization; OR, odds ratio; CI, confidence interval.

factors could adversely impact the study results and even pose a significant threat to MR. Therefore, it is crucial to employ a series of methods to assess whether significant horizontal pleiotropy and heterogeneity exist in the study. The results of Cochran's *Q* test indicated that both the MR-Egger regression and IVW methods yielded no evidence of heterogeneity susceptibility in this study (P>0.05) (*Table 1*). The global test using the MR-PRESSO method did not detect potential outliers with horizontal pleiotropy (P>0.05) (*Table 1*). Additionally, the relationship of the MR-Egger intercept with zero suggested the absence of horizontal pleiotropy in the selected IV SNPs (P>0.05) (*Table 1*). This finding has no significant impact on the causal relationship analysis between exposure factors and outcome variables, consistent with our leave-one-out analysis, forest plots, and scatter plots. This reaffirms the robustness of our research findings (Figures S1,S2).

The reverse MR analysis

When IPF was taken as the exposure variable, and chronic HBV infection and chronic HCV infection were analyzed as outcome variables, the reverse MR analysis using the IVW method in this study indicated that IPF is neither a risk factor for chronic HBV infection (OR =0.986, 95% CI: 0.839–1.159, P=0.87) nor for chronic HCV infection (OR =0.957, 95% CI: 0.856–1.070, P=0.44). Other MR analysis methods, including WM and MR-Egger, also confirmed these results (*Figure 3*, Figures S3,S4).

Discussion

Chronic HBV infection and chronic HCV infection are infectious diseases primarily affecting the liver, caused by HBV and HCV, respectively. They pose significant public health challenges globally due to their predominant impact on liver damage. An estimated approximately 354 million people worldwide are chronically infected with HBV, and around 800,000 deaths occur annually due to diseases related to HBV infection (17). Some observational studies suggest that HBV and HCV are not only the major risk factors for liver fibrosis but also for IPF. Despite inconsistent viewpoints across various studies, the precise relationship needs further exploration from different perspectives.

This study represents the first application of a two-sample bidirectional MR approach to genetically assess the causal associations between HBV and HCV infections and IPF. Our results indicate that there is no clear evidence supporting a causal link between HBV or HCV and an

increased or decreased risk of IPF, and similarly, there is no clear evidence supporting a causal relationship where IPF increases or decreases the risk of HBV or HCV. Compared to previous observational studies, our findings are less likely to be influenced by confounding and reverse causation biases. This conclusion may suggest that viral hepatitis, particularly HBV and HCV, is unlikely to be a causal factor in the development of IPF.

This study has certain limitations. Firstly, because this study used a public database, there was no access to the raw data, resulting in the inability to provide detailed demographic data, which is an important limitation. Indeed, the lack of detailed demographic data may have hampered the interpretation of the findings. Meanwhile, the research is restricted to populations of European ancestry. Although this minimizes bias due to population stratification, the inability to access the raw data and the relatively small sample size prevented stratified analyses according to different age groups. Secondly, since both the case and control groups are of European ancestry, the generalizability of the study results to other ethnic groups needs further evaluation. Additionally, we only conducted an analysis at the level of virus types causing chronic viral hepatitis and did not assess the risk impact of these virus types at the antibody level corresponding to each virus type. Finally, during the screening of IV SNPs closely related to exposure, we set the LD coefficient r^2 to 0.01 and the LD block width to 1,000 kb. Normally, an r^2 of 0.001 and a kb of 10,000 are used to ensure linkage equilibrium between SNPs. However, under the chosen settings, fewer SNPs were selected, potentially impacting the study results to some extent.

Conclusions

This study indicates that there is no causal relationship between chronic HBV and HCV infections at the genetic level and IPF. However, this finding requires support from larger sample-sized GWAS databases for further MR analysis. Additionally, more clinical studies and animal experiments are needed to validate these findings. This is crucial for elucidating the etiology of IPF and for the development of targeted, personalized prevention, and treatment strategies.

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Footnote

Reporting Checklist: The authors have completed the STROBE-MR reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-392/rc

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-392/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-392/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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